The Role of Prognostic variables in the Maturation of ovarian Cancer

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Abstract

Background. Ovarian cancer is the leading cause of death of all gynecological tumors. Most often, ovarian cancer arises from the epithelial layer of the ovary. Most of the cases are identified in highly developed stages.

Methodology. Fifty previously diagnosed patients with ovarian cancer were considered and the venous blood samples of 5 mL were taken, following their informed consent.Fifty healthy women of the same age were regarded as a control group, and blood samples were obtained following their consent. The collected samples were analyzed for the following biomarkers: Malondialdehyde (MDA), Superoxide Dismutase (SOD), Glutathione (GSH), Catalase (CAT), Interleukin-2 (IL-2), Tumor Necrosis Factor-alpha (TNF-α), Matrix Metalloproteinase-9 (MMP-9), and Nitric Oxide (NO).

Results. Serum levels of ovarian cancer patients regarding MDA, TNF-α, MPP-9, and NO were significantly raised as compared to the control. However, the levels of SOD, GSH, and CAT was significantly low.

Conclusion. Highlevels of MDA, TNF-α, MMP-9, and NO, whereas low levels of GSH, CAT, and SOD can be used as an early predictor of ovarian cancer.

Keywords. Ovarian cancer, SOD, MDA, GSH, CAT, IL-2, TNF-α, MPP-9, and NO.

INTRODUCTION

Ovarian cancer is the 3rd most gynecological cancer globally. The risk of ovarian cancer increases with age along with having a family history[1]. It is most frequently called the “silent killer” because of non-specific symptoms. It is usually identified at later stages because of the lack of screening for the early finding and its anatomical location and comparative asymptomatic occurrence[2]. Therefore, the most common cases are identified in a highly developed stage, which contributesto a poor prognosis and is also dependable for the increased burden of the disease[3].

Pakistan has one of the highest rates of ovarian cancer, but the precise occurrence of ovarian cancer is not known, it is the fourth most common cancer among females and is marked to present at the advanced stage[4]. The accurate cause of ovarian cancer in Pakistan is not known, but it has been measured that it is due to the germ line mutation in BRCA1 (Breast cancer gene 1) and BRCA2 (Breast cancer gene 2)genes[5]. According to the Karachi Cancer Registry, ovarian cancer is the third most prevalent malignancy among Pakistani women[6]. Among the South Asian countries, including India, Bhutan, and Bangladesh ovarian cancer is relatively frequent in Pakistan[7].According to World Cancer Research Fund International statistics, Ovarian cancer is the

18th most common worldwide and 8th most common cause of death in women.The ovarian cancer rates are high in China and India[8].

Antioxidants help reduce oxidative stress by neutralizing reactive oxygen species before they can harm biological molecules. They also prevent oxidative damage from spreading by disrupting the chain reactions involved in lipid peroxidation. Lipids are polyunsaturated fatty acids and can easily be attacked by free radicals which can eventually initiate lipid peroxidation. The end product of lipid peroxidation is malondialdehyde (MDA).They can inhibit the action of protective enzymes and act as a tumor promoter and carcinogenic agent. Cell division can be controlled by Lipid peroxidation.High levels of MDA in ovarian cancer can contribute to anincreasein oxidative stress due to the deficiency of antioxidants. Superoxide dismutase (SOD) is an antioxidant enzyme, thatis widely distributed in all cells, especially in the erythrocytes. This enzyme has a crucial role in scavenging superradicals, and protecting cells by dismutating the highly reactive superoxide anion to oxygen and a less reactive oxygen species[9].

AIMS AND OBJECTIVES

The aims and objectives of the present project were:

- **I-** To investigate the relationship between oxidative stress and the influence of external factors in the development of ovarian cancer
- **II-** To investigate the possible mechanisms of action of matrix metalloproteinases (MMPs) and inflammatory cytokines (IL-2 and TNF-α) in the prognosis of ovarian cancer.

MATERIALS AND METHODS

SOURCE OF DATA

The present study was designed to investigate the key processes involved in Matrix metalloproteinase and inflammatory cytokines in the development of ovarian cancer. All the selected patients were screened at Inmol Hospital Lahore. Fifty female patients aged 20-50 years were eligible for inclusion in the study. Informed consent was obtained before being included in this study. Fifty clinically healthy individuals were included as controls. The Research, Ethical Committee of The Institute of Molecular Biology and Biotechnology, The University of Lahore approved the experimental protocol. Five milliliters of venous blood weredrawn from the anti-cubital vein of each participant. The sample bottles were centrifuged within one hour of collection, after which the serum was separated and stored at -70°C until assayed.

INCLUSION CRITERIA

Patients clinically diagnosed with ovarian cancer were included in this study.

EXCLUSION CRITERIA

The subjects witha history of taking drugs (including alcohol and cigarettes), and pre-diagnosis medications (e.g. antiparkinsonian/antipsychotic), were excluded from this study. None of the controls were on any medication, history of chronic infections, malnutrition syndrome, depression, psychosis, or metabolic dysfunction (Such as diabetes mellitus, liver diseases, cancer) that could interfere with their oxidative metabolites and thyroid hormone status.

RESULT

HEMATOLOGICAL PROFILE OF PATIENTS OVARIAN CANCER (STAGE-III)

The data compiled in Table 01 suggests the hematological and demographic profile of ovarian cancer patients as compared to the control group. The mean body weight and age of the patients were78.26±1.17kg and 45.26±6.23yrs, while the mean body weight and age of control individuals were 81.26±1.26kg and

47.26±4.26yrs respectively. The low levels of RBC, Hct, and Hb were observed in ovarian cancer patients (3.36±0.18/L, 44.19±3.09%, and 12.31±2.09g/L) in comparison to control individuals (4.11±1.09/L, 55.06±4.16% and 14.32±1.09g/L) correspondingly. While the mean values of WBC and neutrophil counts were significantly increased in ovarian cancer patients (10.32±2.16/L and 9.16±1.01/L) as compared to control individuals (6.95±2.16/L and 6.17±2.06/L).

TABLE:01	HEMATOLOGICAL PROFILE OF PATIENTS OVARIAN CANCER (STAGE-III)

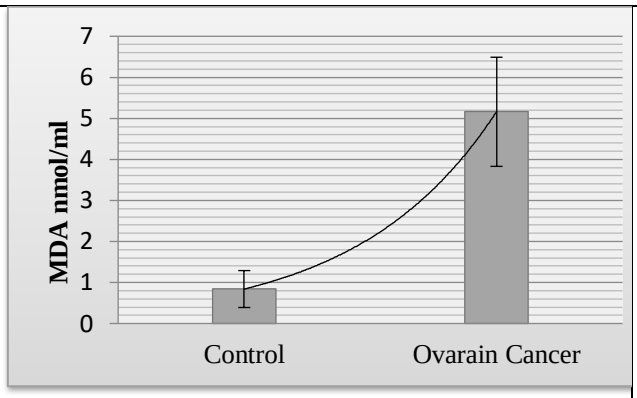
VARIABLES	CONTROL (n=50)	SUBJECTS (n=50)
B. Weight	81.26±1.26	78.26±1.17s
Ageyrs	47.26±4.26	45.26±6.23
RBCs/L	4.11±0.4	3.36±0.18
WBCs/L	6.95±1.16	10.32±2.16
Hct %	55.06±4.16	44.19±3.09
Neutrophil Counts/L	6.17±1.06	9.16±1.01
Hb g/L	14.32±1.09	12.31±2.09

EXPRESSION OF PROGNOSTIC VARIABLES OF MEDICAL IMPORTANCE AND THEIR IMPULSIVE INTERPLAY TO DEVELOP OVARIAN CANCER(STAGE III)

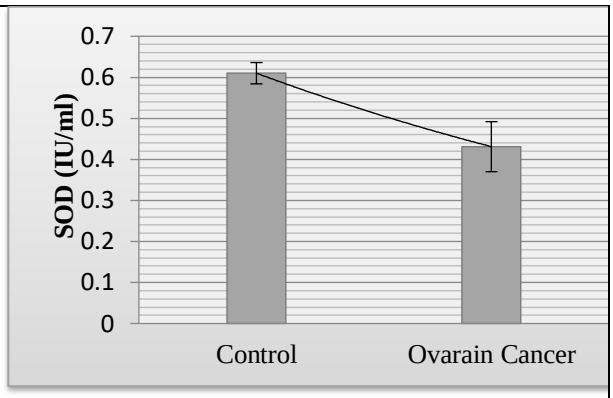
The data presented in Table 02 shows the prognostic variables of medical importance and their impulsive interplay in the development of ovarian cancer. Malondialdehyde (MDA) which is the end product of lipid peroxidation, has a significant difference between the values of control and patients with ovarian cancer. The mean value of MDA was significantly increased in ovarian cancer patients (5.16±1.33 nmol/ml) as compared to control individuals(0.84±0.45nmol/ml). The mean value of NO was also to be raised in ovarian cancer subjects (49.36±8.16µmol/L) in comparison to control persons (19.65±2.18µmol/L). On the other hand, the levels of anti-oxidants were significantly decreased in ovarian cancer subjects as compared to control persons. The mean value of SOD, GSH, and CAT was significantly reduced in the patients with ovarian cancer (0.431±0.061IU/ml, 4.09±2.01µmol/L, and 3.954±0.656IU/L) as compared to control individuals (0.61±0.026IU/ml, 8.16±1.01µmol/L and 6.55±1.52IU/L) respectively. Significantly increased levels of IL-2, TNF-α and MMP-9 were observed in ovarian cancer patients (16.35±2.39pg/ml, 37.26±4.26pg/ml and 66.35±3.88ng/ml) as compared to control (21.26±2.01pg/ml, 16.35±3.88pg/ml and 42.16±8.16ng/ml) which are responsible for inflammation and ECM degradation.

TABLE 02: EXPRESSION OF PROGNOSTIC VARIABLES OF MEDICAL IMPORTANCE AND THEIR IMPULSIVE INTERPLAY TO DEVELOP OVARIAN CANCER(STAGE-III)

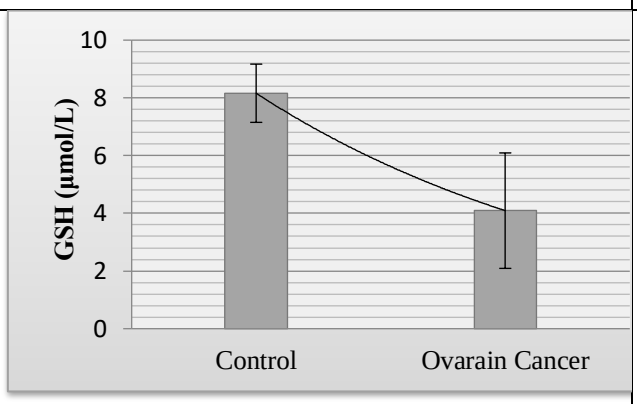
VARIABLES	CONTROL (n=50)	SUBJECT (n=50)	*P*-VALUE (0.05)
MDA nmol/ml	0.84±0.45	5.16±1.33	0.015
SOD (IU/ml)	0.61±0.026	0.431±0.061	0.001
GSH (µmol/L)	8.16±1.01	4.09±2.01	0.031
CAT (IU/L)	6.55±1.52	3.954±0.656	0.041
IL-2 (pg/ml)	21.26±2.01	16.35±2.39	0.000
TNF-α (pg/ml)	16.35±3.88	37.26±4.26	0.021
MPP-9 (ng/ml)	42.16±8.16	66.35±3.88	0.019
NO (µmol/L)	19.65±2.18	49.36±8.16	0.008


MDA nmol/ml
7
6
5
4
3
2
1
0
Control
Ovarain Cancer

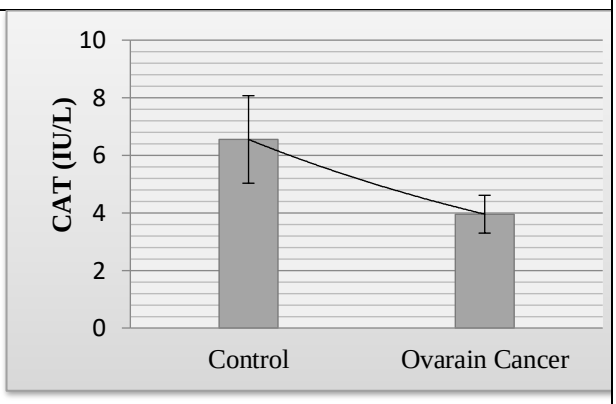
A


SOD (IU/ml)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Control
Ovarain Cancer

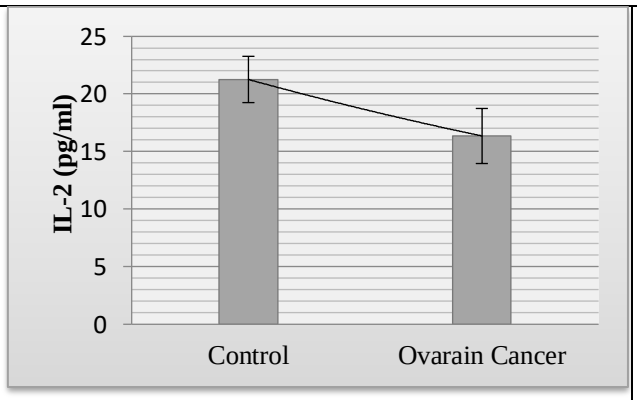
B


GSH (µmol/L)
10
8
6
4
2
0
Control
Ovarain Cancer

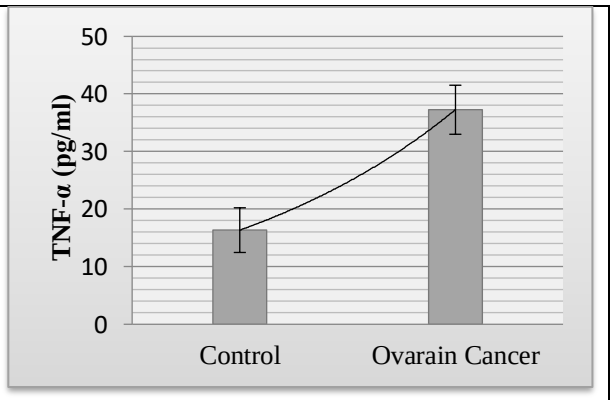
C


CAT (IU/L)
10
8
6
4
2
0
Control
Ovarain Cancer

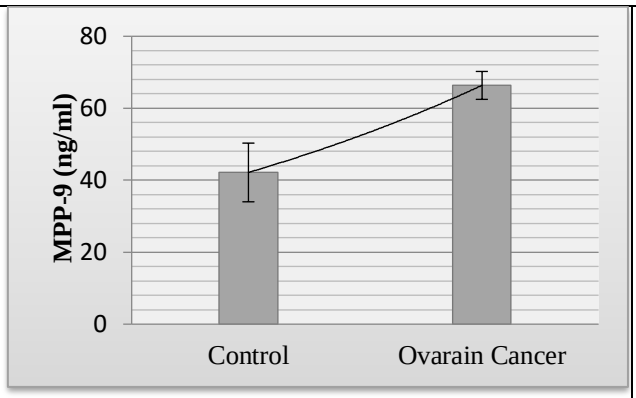
D


IL-2 (pg/ml)
25
20
15
10
5
0
Control
Ovarain Cancer

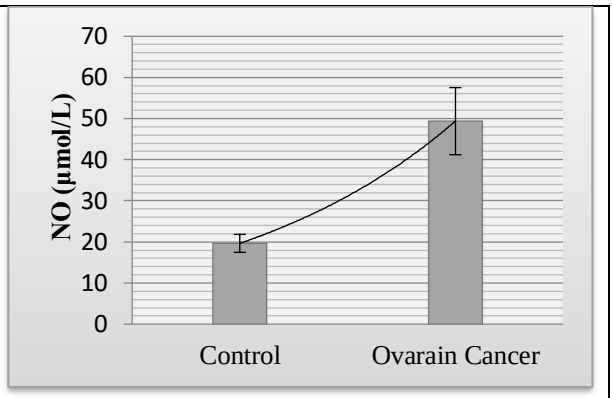
E


TNF-α (pg/ml)
50
40
30
20
10
0
Control
Ovarain Cancer

F



G



H

TABLE 02: EXPRESSION OF PROGNOSTIC VARIABLES OF MEDICAL IMPORTANCE AND THEIR IMPULSIVE INTERPLAY TO DEVELOP OVARIAN CANCER(STAGE-III)

DISCUSSION

Ovarian cancer is a diverse and aggressive group of diseases in both pre-menopausal and post-menopausal women, with more than 80% of all cases being diagnosed in women over the age of 50 years. Ovarian cancer is the fifth most common cancer in women in the developing world and is most frequently called the “silent killer” due to its non-specific symptoms. It is typically diagnosed at an advanced stageonce the tumor has progressed significantly. Unfortunately, effective preventive measures and reliable screening tools for early detection are still unavailable. The research has shown that factors such as inflammation, excessive ovulation, elevated steroid hormones, obesity, age, infertility, and certain reproductive factors like nulliparity increase the risk of ovarian cancer. While appropriate surgery and effective chemotherapy can treat this disease, early diagnosis of ovarian malignancy is crucial for improving prognosis. In recent years, it has been visible that free radicals, including reactive oxygen species (ROS),plays a significant role in the development of ovarian cancer. Various biochemical and physiological processes in the human body produce ROS as a by-product. Therefore, this study was conducted to evaluate the role of different matrix metalloproteinase (MMP), oxidative stress parameters, profile of antioxidants, and inflammatory markers in ovarian cancer.

Lipids are particularly known as polyunsaturated fatty acids,which are quite susceptible to free radicals thatinitiate lipid peroxidation. One of the end products of lipid peroxidation is malondialdehyde (MDA), formed through the decomposition of arachidonic acid and other larger polyunsaturated fatty acids (PUFAs)[10]. MDA is known for its inhibitory effect, acting both as a tumor promoter and carcinogenic agent. The significance of lipid peroxidation indicates that it plays an important role in cell proliferation and division. Elevated levels of MDA in ovarian cancer areare linked to increased oxidative stress especially when there is a deficiency of antioxidants, which help to defend against oxidative damage. In this study, MDA levels were found to be significantly higher in ovarian cancer patients. A significant increase in MDAlevelsamong our patients is indicative of elevated oxidative stress and a higher generation of reactive oxygen species. Similar findings were reported in the work of Nayak*et al*[11] and Kumaraguruparan*et al*[12],who also observed an increase in MDA levels in ovarian cancerpatients. In the present study, there is a highly significant negative correlation was found between MDA and SOD,indicating that decreased

antioxidantlevels contribute to enhance lipid peroxidation (MDA vs. SOD, r= -0.756**).

Nitric oxide (NO) is an endogenous gas molecule with a short life span that serves multiple functions. It is a highly reactive free radical that is produced from the molecular oxygen and NADPH by nitric oxide synthase (NOS) and 1-arginine. Nitric oxide regulatesvarious physiological and pathophysiological processes, including vasodilation, blood flow, angiogenesis, vascular permeability, respiration, immune response, cell migration, apoptosis and neurological functions such as neurotransmission and development of the nervous system[13]. Nitric oxide has a dual rolein tumor progression, both promoting and inhibiting tumor growth and metastasis. Elevated and uncontrolled levels of nitric oxide can leadtocell death and contribute to various illness[14]. The expression of Nitric oxide and nitric oxide synthase is observed in numerous human tumors, with levels varying based on tumor type and stage. The research carried out by Dai *et al*[15]found that the expression levels of NO and NOS in many tumors increased as compared to normal tissues. The impact of nitric oxide on tumor progression depends upon its source either from tumor cell or stromal cell, as well as the type and activity of tumor nitric oxide synthase. In the present study, we found that the mean value of NO was significantly elevated in ovarian cancer patients as compared to healthy individuals. Additionally, a strong negative correlation was observed between NO and SOD (NO vs. SOD, r= -0.812*),suggesting thatdecreased levels of antioxidants may lead to enhance nitric oxide species.

Glutathione (GSH) is a tripeptide that is composed of cysteine, glutamic acid, and glycine. It plays animportant role as an antioxidant in various cellular functions, including cell differentiation, proliferation and apoptosis, and nutrient metabolism. GSH is the primary thioltripeptide produced by the liver and is integral to the cellular defense mechanism[16]. It helps eliminate toxins from the liver, red blood cells, lungs, and intestinal tract and neutralizes a wide range of harmful substances, including those from cigarette smoke, heavy metals, radiation, alcohol, and cancer chemotherapy.

Additionally, GSH protects cells by neutralizing reactive oxygen species before they can cause damage to the cell. A deficiency in Glutathione can lead to oxidative stress, contributing to the aging and progression of many diseases including cancer. Free radicals generated by reactive oxygen species in cancer cells result in the accumulation of oxidative products in DNA, proteins, and lipids[17]. The maintenance of appropriate levels of glutathione and oxidation state is vital for normal cellular functions, while imbalances can lead to various diseases, including cancers[18]. In the present study, we found that the mean value of GSH was lower in ovarian cancer patients as compared to healthy individuals, consistent with findings from Maher[19]. Additionally, GSH showed a statistically significant negative correlation with NO (r = 0.320*).

Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases produced by cells according to the cells required by wound cells as well as inflammatory cells. They play an important role in both normal and pathological conditions. Under normal circumstances, MMPs are produced in very low amounts and are involved in tissue remodeling processes like ossification, embryonic development, placental development, and wound healing, However, in disease conditions,dysregulation can lead to increasedlevels of MMPs, which are implicated in rheumatoid arthritis and various cancers. The activation of MMPs can be induced by inflammatory growth factors, cytokines, and cell stroma interaction, particularly in most malignant cells[20]. Conversely, MMPs can be inactivated by various inhibitors, such as tissue inhibitors of metalloproteinases (TIMPs) and α-2 macroglobulin, or by synthetic matrix metalloproteinase inhibitors (MMPIs).MMP-9alsoknown as gelatinases B is involved in tumor development and genetic damage, contributing to the tumorigenicity, neoplastic extension,

accretion of mutation, tumor-associated angiogenesis, increased survival, adhesive interaction, matrix degradation, and the loss of basement membrane, motility, tumor cell invasion and the regulation of cancer stem cell [21]. The research carried out by Tomg*et al*[22] and Kenny *et al*[23], established a strong associationbetweenMMP-9 and gynecological tumors,finding that MMP-9 levels were significantly higher in ovarian cancer patients as compared to control subjects, particularly in advanced stages of the disease with metastasis.

Interleukin-2 (IL-2) is a versatile pleiotropic cytokine that influences cell growth and differentiation by regulatingthe expression of genes encoding growth factors, cellular receptors, and other cytokines. As a diverse proinflammatory cytokine, IL-2plays several biological functions, including promoting the inflammatory response, maintaining cellular immunity, and providing host defense against infection[24]. It is produced in a wide range of proinflammatory cytokines, which can be tightly regulated. It acts as both an autocrine and paracrine growth factor, promoting malignant progression, but its expression is elevated in response to infection or injury and macrophages[25].

Tumor necrosis factor alpha (TNF-α) belongs to the superfamily of TNF/TNFR,playing a key role in maintaining immune system homeostasis, inflammation, and host defense. TNF-α is involved in various pathological processes, including autoimmunity, malignancy, and chronic inflammation. It is frequently detected in biopsies of human cancer, which is formed by both epithelial tumor cells such as ovarian and renal cancer, or stromal cells like in breast cancer[26].

The research carried out by Naylor *et al*[27]demonstrated that TNF-α plays a significant role in the development of ovarian cancer and its receptors are also articulated in the ovaries of females during a cancer condition. Moreover, the lower level of tumor necrosis factor-α in ovarian cancer patients can help reduce the tumor growth, development, metastasis, epithelial cell expansion, and fabrication of tumor-promoting chemokines and cytokines. Conversely, elevated levels of TNF-α can enhance tumor development and invasion by stimulating the secretion of cytokines, matrix metalloproteinase (MMP), and proangiogenic factors. In the present study,we found that levels of TNF-α were significantly higher in ovarian cancer patients as compared to healthy individuals[28].

Liu Set al[29]discussed research advancements in prognostic factors and biomarkers of ovarian cancer. The increased levels of the antioxidant 8-OHdG (8-Hydroxy Guanosine) are associated with poor prognosis in the immune response. The increased TNFa/IL-4 ratio has a good prognosis.Waki K et al[29] reported that the CD4/CD8 ratio (CD stands for Cluster of Differentiation, which is a transmembrane glycoprotein coreceptor for the T cell) serves as a good prognostic factor for ovarian cancer patients. The decreased ratio of CD4/CD8 suggests a favorable prognosis.Shao et al[30]examined prognostic factors and clinicopathological characteristics of ovarian tumors and concluded that elevated CA 125 levels lead to poor prognosis.

CONCLUSION:

In ovarian cancer patients, levels of MDA, TNF-α, MMP-9, and NO are elevated, whereas GSH, CAT and SOD levels are reduced. Estimation of these biomarkers could serve as early predictor of ovarian cancer.

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